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Abstract

The purpose of this study was to investigate the effects of topical treatment with zinc oxide (2.5%, 10%, 25% and 50%) and intraperitoneal treatment with diethyldithiocarbamate (DEDTC) (50 mg/kg, 500 mg/kg and 1,000 mg/kg) on the mitotic index of epidermal basal cells in incised and non-incised mouse skin. The present results showed that topical application of zinc oxide (25% and 50%) increased the mitotic index of epidermal basal cells in incised skin and non-incised skin. Conversely, intraperitoneal administration of DEDTC (500 mg/kg and 1,000 mg/kg) decreased the mitotic index, but only in the incised skin. These results suggest that mitosis of epidermal basal cells may be stimulated by the topical application of zinc oxide both in incised and non-incised mouse skin, and that it also may be inhibited by the intraperitoneal administration of DEDTC in incised mouse skin.

KEYWORDS: zinc oxide, mitotic index, epidermal basal cells, mouse skin

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The purpose of this study was to investigate the effects of topical treatment with zinc oxide (2.5%, 10%, 25% and 50%) and intraperitoneal treatment with diethyldithiocarbamate (DEDTC) (50 mg/kg, 500 mg/kg and 1,000 mg/kg) on the mitotic index of epidermal basal cells in incised and non-incised mouse skin. The present results showed that topical application of zinc oxide (25% and 50%) increased the mitotic index of epidermal basal cells in incised skin and non-incised skin. Conversely, intraperitoneal administration of DEDTC (500 mg/kg and 1,000 mg/kg) decreased the mitotic index, but only in the incised skin. These results suggest that mitosis of epidermal basal cells may be stimulated by the topical application of zinc oxide both in incised and non-incised mouse skin, and that it also may be inhibited by the intraperitoneal administration of DEDTC in incised mouse skin.

Key words: zinc oxide, mitotic index, epidermal basal cells, mouse skin

It is well known that in humans about 20 % of total body zinc is present in the skin and the corresponding figure for rats is about 38 % (1). Zinc is an essential cofactor for many enzymes important in skin physiology and is also involved in the processes of keratinization (2) and wound healing (3-5). In animals, the manifestations of zinc deficiency include loss of hair, thickening and hyperkeratinization of the epidermis, and orally administered zinc salts improve the condition of the skin (6). In clinical study, a severe deficiency of zinc has been reported in patients with acrodermatitis enteropathica (AE) and intravenous hyperalimentation therapy without zinc supplementation (6). A reduced zinc content of skin has also been found in patients with psoriasis (7, 8), acne, dermatitis herpetiformis and Darier's disease (9). Many studies reported that the process of wound healing is promoted by zinc supplementation (3, 5, 10, 11). Diethyldithiocarbamate (DEDTC), a metal chelating agent, is known to reduce the concentration of zinc in brain tissue (12).

The following study was conducted to investigate the effects of topical application of zinc oxide (2.5 %, 10 %, 25 % and 50 %) and intraperitoneal administration of DEDTC (50 mg/kg, 500 mg/kg and 1,000 mg/kg) on the mitotic index (MI) of epidermal basal cells in incised and non-incised mouse skin.

Materials and Methods

Adult ddN strain mice, weighing 24-26 g at the beginning of the experiment, were used in this study. The mice were housed under natural light and maintained at 22 ± 2 °C. They were kept in cages with free access to tap water. The commercial stock food (Oriental Yeast Co., Ltd., Tokyo, Japan) was provided to these mice. The following drugs were used: zinc oxide in white vaseline (2.5 %, 10 %, 25 % and 50 % zinc oxide topical application) and DEDTC solution (50 mg/kg, 500 mg/kg and 1,000 mg/kg i.p.) (13).

All mice were anesthetized with ethyl ether and positioned in a stereotaxic instrument. The middle dorsal skin about 2 × 2 cm was shaved with hair remover cream (EBA^R cream, Tanabe-Seiyaku, Ltd., Tokyo, Japan) and cleaned with Isodine^R solution (Meigi-Seika, Ltd., Tokyo, Japan). Then the mice were divided into the non-incised and incised skin groups. In the incised skin group, two incisions 5 mm in length were made on the shaved area of

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Table I Experimental design and treatment procedures

Group	No. of mice	Treatment procedures	Days of treatment
Non-incised skin			
Control	10	Topically applied with white vaseline, once a day	5
2.5% zinc oxide	8	Topically applied with 2.5% zinc oxide, once a day	4
10% zinc oxide	8	Topically applied with 10% zinc oxide, once a day	4
25% zinc oxide	8	Topically applied with 25% zinc oxide, once a day	4
50% zinc oxide	8	Topically applied with 50% zinc oxide, once a day	4
DEDTC (50mg/kg)	8	i.p. injection with 50mg/kg of DEDTC, and topically applied with white vaseline, once a day	4
DEDTC (500mg/kg)	8	i.p. injection with 500mg/kg of DEDTC, and topically applied with white vaseline, once a day	
DEDTC (1000mg/kg)	8	i.p. injection with 1000mg/kg of DEDTC, and topically applied with white vaseline, once a day	4
Incised skin			
Control	26	Topically applied with white vaseline, once a day	13
2.5% zinc oxide	8	Topically applied with 2.5% zinc oxide, once a day	4
10% zinc oxide	8	Topically applied with 10% zinc oxide, once a day	4
25% zinc oxide	24	Topically applied with 25% zinc oxide, once a day	12
50% zinc oxide	8	Topically applied with 50% zinc oxide, once a day	4
DEDTC (50mg/kg)	8	i.p. injection with 50mg/kg of DEDTC, and topically applied with white vaseline, once a day	4
DEDTC (500mg/kg)	24	i.p. injection with 500mg/kg of DEDTC, and topically applied with white vaseline, once a day	12
DEDTC (1000mg/kg)	8	i.p. injection with 1000mg/kg of DEDTC, and topically applied with white vaseline, once a day	4

each mouse, and the distance between the two incisions was 1cm. The incisions were closed with interrupted sutures. Table 1 shows the treatment procedures in the two groups. In both the treatment and control groups, four biopsies from two mice were taken every day at 10: 00 during the treatment period. The biopsies were fixed with Carnoy's fixative, dehydrated, and embedded in paraffin. Each biopsy was cut into 8–10 serial sections at a thickness of 4 μm, and finally stained with hematoxylin and eosin according to routine techniques.

The MI was defined as the number of mitotic cells per 100 epidermal basal cells of mouse skin. The results of MI are presented as mean ± S.E.M. (%). Each mean value was based on observation of at least 32 histologic sections from four biopsies of two mice.

Results

Table 2 shows the MI of epidermal basal cells in control skin and non-incised skin with treatment of different doses of zinc oxide and DEDTC. The MI was significantly increased with the application of 25 % and 50 % zinc oxide as compared with control ($P < 0.001$ or $P < 0.01$), from the 1st day on. It was mildly increased with treatment of 10 % zinc oxide only on the 2nd day ($P < 0.1$). The MI was not affected by treatment with 2.5 % zinc oxide or with three doses of DEDTC.

Table 3 shows the MI of epidermal basal cells in control incised skin and incised skin treated with different doses of zinc oxide and DEDTC. In control incised skin, the MI continuously increased and appeared to be maximal on the third day, followed by a gradual decrease. On the third day, the MI was influenced by treatment with different dose of zinc oxide and DEDTC (see Fig. 1). When treated with 25 % zinc oxide or 50 % zinc oxide, the MI was significantly increased compared with the control ($P < 0.001$); whereas, when treated with 500 mg/kg or 1,000mg/kg of DEDTC, the MI was significantly decreased compared with the control ($P < 0.001$), higher dose (1,000mg/kg of DEDTC) producing a more pronounced effect. When treated with 2.5 % and 10 % zinc oxide or 50mg/kg of DEDTC, the MI was

not changed compared with the control.

Our results also showed that, following treatment of 25% zinc oxide, the number of mitotic cells was increased both in the non-incised skin and in the incised skin as compared with that in normal mouse skin. Fig. 2 shows a light microscopic view of normal mouse skin.

Table 2 The mitotic index of epidermal basal cells in non-incised mouse skin

Group	Mitotic index after treatment (%)				
	0 day	1st day	2nd day	3rd day	4th day
Control	2.0 ± 0.07	1.9 ± 0.04	1.9 ± 0.08	2.1 ± 0.02	2.0 ± 0.07
2.5% zinc oxide	—	2.1 ± 0.05	2.3 ± 0.09	2.3 ± 0.12	2.2 ± 0.08
10% zinc oxide	—	2.1 ± 0.05	2.4 ± 0.09***	2.3 ± 0.12	2.2 ± 0.08
25% zinc oxide	—	2.5 ± 0.12**	3.6 ± 0.14*	4.0 ± 0.10*	4.0 ± 0.08*
50% zinc oxide	—	3.2 ± 0.14*	3.5 ± 0.09*	4.1 ± 0.11*	4.0 ± 0.08*
50 mg/kg DEDTC	—	2.1 ± 0.04	1.9 ± 0.07	2.0 ± 0.06	2.1 ± 0.06
500 mg/kg DEDTC	—	2.0 ± 0.07	2.0 ± 0.04	2.0 ± 0.08	1.9 ± 0.06
1000 mg/kg DEDTC	—	1.9 ± 0.07	1.8 ± 0.04	1.9 ± 0.06	1.9 ± 0.06

*$P < 0.001$, **$P < 0.01$, ***$P < 0.1$ *versus* control. Values are mean ± S.E.M.

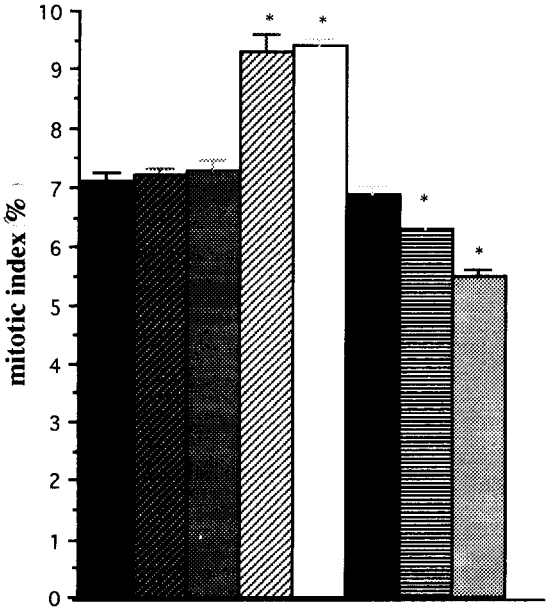


Fig. 1 The mitotic index of epidermal basal cells in incised mouse skin treated with different doses of zinc oxide or diethyldithiocarbamate (DEDTC) and in control, on the 3rd day. Each point represents the mean ± S.E.M. (■) control, (▨) 2.5% zinc oxide, (▩) 10% zinc oxide, (▨) 25% zinc oxide, (□) 50% zinc oxide, (▩) DEDTC (50 mg/kg), (▤) DEDTC (500 mg/kg), (▦) DEDTC (1000 mg/kg). *$P < 0.001$.

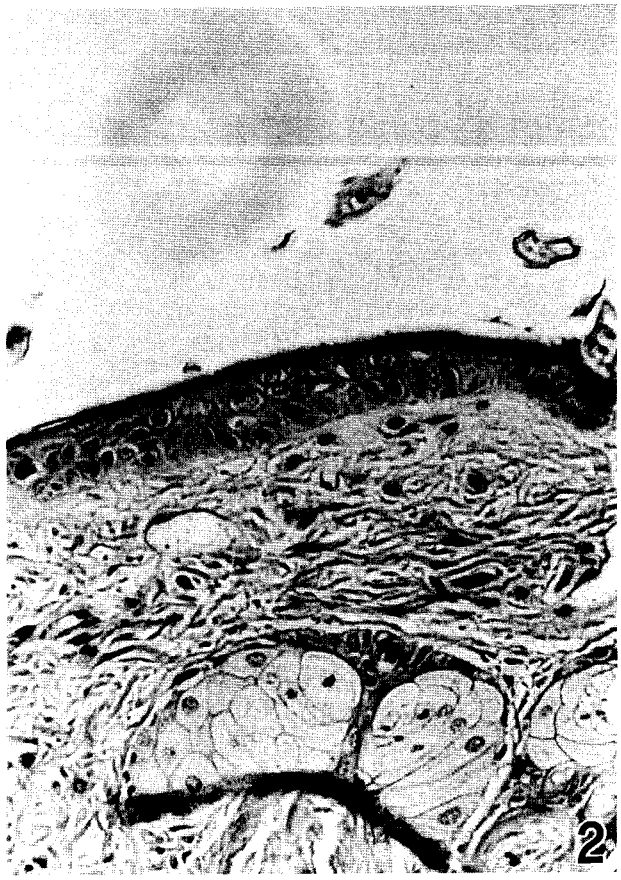
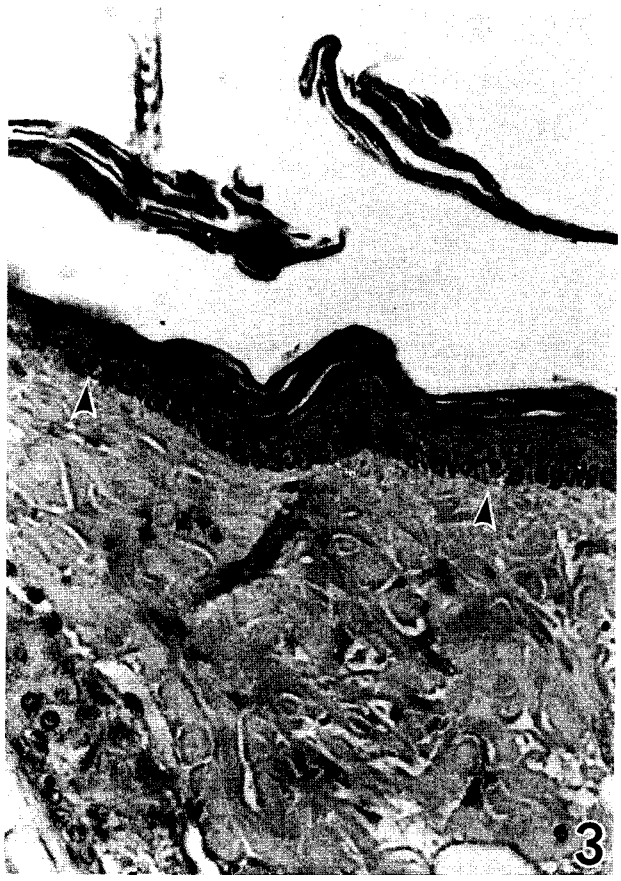


Fig. 2 Light microscopic view of normal mouse skin.

Table 3 The mitotic index of epidermal basal cells in incised mouse skin

Group	Mitotic index after treatment (%)												
	0 day	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day
Control	2.0 ± 0.07	3.2 ± 0.12	5.0 ± 0.15	7.1 ± 0.17	4.8 ± 0.15	3.2 ± 0.15	3.0 ± 0.11	2.7 ± 0.10	2.6 ± 0.07	2.4 ± 0.07	2.0 ± 0.08	1.9 ± 0.05	2.0 ± 0.07
2.5% zinc oxide	—	—	5.1 ± 0.10	7.2 ± 0.12	5.0 ± 0.13	3.3 ± 0.15	—	—	—	—	—	—	—
10%zinc oxide	—	—	5.3 ± 0.10	7.3 ± 0.17	5.3 ± 0.13	3.4 ± 0.17	—	—	—	—	—	—	—
25% zinc oxide	—	3.5 ± 0.10	6.5 ± 0.13*	9.3 ± 0.25*	6.4 ± 0.14*	5.6 ± 0.16*	5.1 ± 0.18*	4.7 ± 0.13*	4.0 ± 0.13*	3.8 ± 0.11*	3.7 ± 0.13*	3.9 ± 0.17*	3.8 ± 0.15*
50% zinc oxide	—	—	6.7 ± 0.10*	9.4 ± 0.14*	6.4 ± 0.11*	5.5 ± 0.10*	—	—	—	—	—	—	—
50mg/kg DEDTC	—	—	5.1 ± 0.10	7.0 ± 0.13	4.9 ± 0.11	3.1 ± 0.14	—	—	—	—	—	—	—
500mg/kg DEDTC	—	3.0 ± 0.11	4.3 ± 0.15*	6.3 ± 0.15*	5.8 ± 0.18*	4.2 ± 0.11*	3.6 ± 0.12*	3.2 ± 0.13*	3.0 ± 0.12**	2.9 ± 0.12**	2.5 ± 0.01*	2.4 ± 0.01*	2.0 ± 0.01
1000mg/kg DEDTC	—	—	3.8 ± 0.14*	5.5 ± 0.11*	5.6 ± 0.17*	4.1 ± 0.11*	—	—	—	—	—	—	—

*$P < 0.001$, **$P < 0.01$ *versus* control. Values are mean ± S.E.M. DEDTC: diethyldithiocarbamate



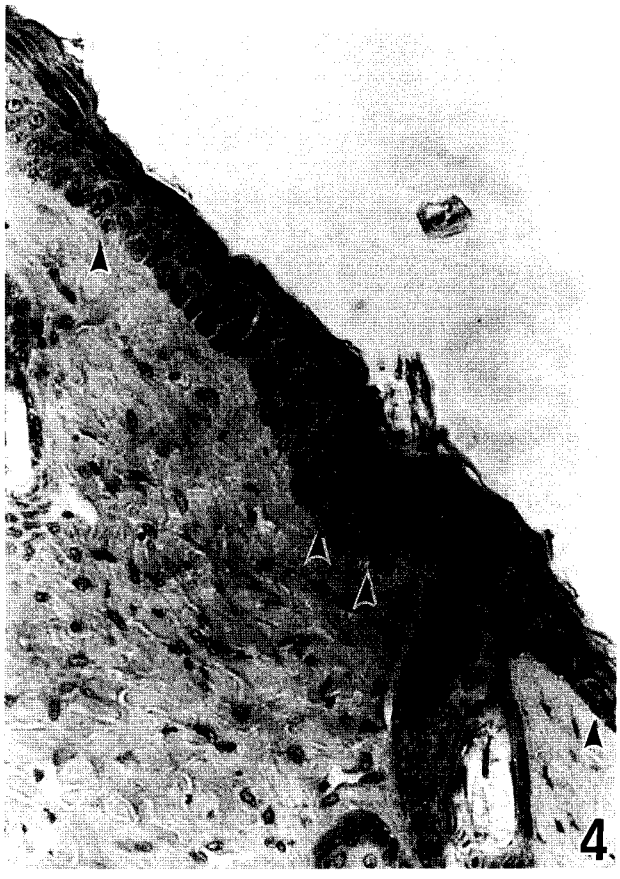


Fig. 3 shows the mitotic cells in non-incised skin with treatment of 25 % zinc oxide. Fig. 4 shows the mitotic cells in incised skin with treatment of 25 % zinc oxide.

Discussion

In this investigation, the effects of treatment with zinc oxide and DEDTC on the MI of epidermal basal cells of mouse skin were assessed. The present finding that the MI was increased by treatment with 25 % and 50 % zinc oxide compared with the control, suggests that zinc oxide promotes mitosis of epidermal basal cells in both incised and non-incised mouse skin. In previous studies, rapid cell division in wounds was connected with zinc levels (2, 5). In a study using radioisotopes, zinc was preferentially concentrated at wound sites, and the zinc concentration appeared to be maximal between 48 and 72 h after injury (14). A mitogenic effect of zinc (at 5 μg zinc/ml) on the epithelial cells *in vitro* has also been demonstrated (15).

The exact mechanisms of the effect of zinc on wound healing are still unclear (16). Zinc is necessary in several deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymerases and transferases (17–20), and a zinc-deficient state will inhibit mitosis (21). Fuji previously reported that zinc was involved at each stage of mitosis and that it also influenced cell division through an interaction with the tubulin in spindles (22). Oteiza *et al.* investigated the hypothesis that abnormal cellular tubulin polymerization may be a biochemical lesion underlying some of the defects associated with zinc deficiency (23). Zinc, at physiological concentrations, stimulates *in vitro* tubulin assembly into microtubules with normal morphological characteristics (24), and tubulin assembly has been reported to be impaired in the brain of zinc-deficient adult rats (25, 26). We suggest that the appearance of mitotic apparatus may have a connection with zinc since the MI was increased by treatment with zinc oxide and decreased by the chelating effect of DEDTC on zinc. It has been observed that the addition of a chelator to various cell culture systems reduces DNA synthesis and that this can be reversed only by the addition of Zn^{2+} to the cultures (27). In the present study, the decrease in the MI after administration of DEDTC in incised mouse skin was most probably caused by the reduction of skin zinc content through chelation.

In conclusion, these findings suggest that mitosis of epidermal basal cells may be promoted by the topical application of zinc oxide in both incised and non-incised mouse skin, and it may be inhibited by the intraperitoneal administration of DEDTC in incised mouse skin.

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Fig. 3 Light microscopic view of non-incised mouse skin section after treatment with 25% zinc oxide. Arrowheads indicate cells undergoing mitosis.

Fig. 4 Light microscopic view of incised mouse skin section after treatment with 25% zinc oxide. Cell division of epidermal cells results in epidermal hyperplasia. Arrowheads indicate cells undergoing mitosis.

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